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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/620,915	07/17/2003	Andrea Lynn Peticca		8943

7590 06/13/2007

Andrea Lynn Peticca
PMB #309
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EXAMINER	
ANDERSON, HEATHER L	
ART UNIT	**PAPER NUMBER**
1609	

MAIL DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/620,915	PETICCA, ANDREA LYNN	
	Examiner	Art Unit	
	Heather Anderson	1609	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 July 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Specification

Content of Specification

The following guidelines illustrate the preferred layout for the specification of a utility application. These guidelines are suggested for the applicant's use. While some of these headings are used in the applicant's specification, the content found therein is not of the appropriate scope and depth required to sufficiently describe the invention. See rejections under 35 U.S.C. 112, first paragraph in the next section of the Office Action.

(a) Title of the Invention: See 37 CFR 1.72(a) and MPEP § 606. The title of the invention should be placed at the top of the first page of the specification unless the title is provided in an application data sheet. The title of the invention should be brief but technically accurate and descriptive, preferably from two to seven words may not contain more than 500 characters.

(b) Cross-References to Related Applications: See 37 CFR 1.78 and MPEP § 201.11. Please note that the instantly recited discussion concerning a "patent reference related to the present marinade was found in a 20 year search of the U.S. Patent and Trademark Office patents" does not constitute a proper cross-reference citation within this section (again, see 37 CFR 1.78 and MPEP § 201.11).

(c) Statement Regarding Federally Sponsored Research and Development: See MPEP § 310.

(d) The Names Of The Parties To A Joint Research Agreement: See 37 CFR 1.71(g).

(e) Incorporation-By-Reference Of Material Submitted On a Compact Disc: The specification is required to include an incorporation-by-reference of electronic documents that are to become part of the permanent United States Patent and Trademark Office records in the file of a patent application. See 37 CFR 1.52(e) and MPEP § 608.05. Computer program listings (37 CFR 1.96(c)), "Sequence Listings" (37 CFR 1.821(c)), and tables having more than 50 pages of text were permitted as electronic documents on compact discs beginning on September 8, 2000.

(f) Background of the Invention: See MPEP § 608.01(c). The specification should set forth the Background of the Invention in two parts:

(1) Field of the Invention: A statement of the field of art to which the invention pertains. This statement may include a paraphrasing of the applicable U.S. patent classification definitions of the subject matter of the claimed invention. This item may also be titled "Technical Field."

(2) Description of the Related Art including information disclosed under 37 CFR 1.97 and 37 CFR 1.98: A description of the related art known to the applicant and including, if applicable, references to specific related art and problems involved in the prior art which are solved by the applicant's invention. This item may also be titled "Background Art."

(g) Brief Summary of the Invention: See MPEP § 608.01(d). A brief summary or general statement of the invention as set forth in 37 CFR 1.73. The summary is separate and distinct from the abstract and is directed toward the invention rather than the disclosure as a whole. The summary may point out the advantages of the invention or how it solves problems previously existent in the prior art (and preferably indicated in the Background of the Invention). In chemical cases it should point out in general terms the utility of the invention. If possible, the nature and gist of the invention or the inventive concept should be set forth. Objects of the invention should be treated briefly and only to the extent that they contribute to an understanding of the invention.

(h) Brief Description of the Several Views of the Drawing(s): See MPEP § 608.01(f). A reference to and brief description of the drawing(s) as set forth in 37 CFR 1.74.

(i) Detailed Description of the Invention: See MPEP § 608.01(g). A description of the preferred embodiment(s) of the invention as required in 37 CFR 1.71. The description should be as short and specific as is necessary to describe the invention adequately and accurately. Where elements or groups of elements, compounds, and processes, which are conventional and generally widely known in the field of the invention described and their exact nature or type is not necessary for an understanding and use of the invention by a person skilled in the art, they should not be described in detail. However, where particularly complicated subject matter is involved or where the elements, compounds, or processes may not be commonly or widely known in the field, the specification should refer to another patent or readily available publication which adequately describes the subject matter.

(j) Claim or Claims: See 37 CFR 1.75 and MPEP § 608.01(m). The claim or claims must commence on separate sheet or electronic page (37 CFR 1.52(b)(3)). Where a claim sets forth a plurality of elements or steps, each element or step of the claim should be separated by a line indentation. There may be plural indentations to further segregate subcombinations or related steps. See 37 CFR 1.75 and MPEP § 608.01(i)-(p).

(k) Abstract of the Disclosure: See MPEP § 608.01(f). A brief narrative of the disclosure as a whole in a single paragraph of 150 words or less commencing on a separate sheet following the claims. In an international application which has entered the national stage (37 CFR 1.491(b)), the applicant need not submit an abstract commencing on a separate sheet if an abstract was published with the international application under PCT Article 21. The abstract that appears on the cover page of the pamphlet published by the International Bureau (IB) of the World Intellectual Property Organization (WIPO) is the abstract that will be used by the USPTO. See MPEP § 1893.03(e).

(l) Sequence Listing, See 37 CFR 1.821-1.825 and MPEP §§ 2421-2431. The requirement for a sequence listing applies to all sequences disclosed in a given application, whether the sequences are claimed or not. See MPEP § 2421.02.

Claim Rejections - 35 USC § 112

The following is an excerpt of MPEP (Manual of Patent Examining Procedure) 2162:

> To obtain a valid patent, a patent application must be filed that contains a full and clear disclosure of the invention in the manner prescribed by 35 U.S.C. 112, first paragraph. The requirement for an adequate disclosure ensures that the public receives something in return for the exclusionary rights that are granted to the inventor by a patent. The grant of a patent helps to foster and enhance the development and disclosure of new ideas and the advancement of scientific knowledge. Upon the grant of a patent in the U.S., information contained in the patent becomes a part of the information available to the public for further research and development, subject only to the patentee's right to exclude others during the life of the patent.
>
> In exchange for the patent rights granted, 35 U.S.C. 112, first paragraph, sets forth the minimum requirements for the quality and quantity of information that must be contained in the patent to justify the grant.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a **written description** of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to **make and use** the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1 – 3 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the **written description requirement**. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The specification is what provides the support for what is being claimed. It must thoroughly describe what the invention is and why the invention is novel, usually with detailed information on the scientific and/or medical facts related to the invention. In applications for patents on compositions, for example, a discussion of each of the various ingredients, including what properties they possess that are important to the invention as a whole and the quantities of each used, is given in the specification.

Applicant's specification gives very limited information and only vague directions. Although the claims themselves are exceedingly unclear (see rejection under 35 U.S.C. 112, second paragraph below), there is no support for what appears to be claimed in the Applicant's specification and is therefore rejected on the grounds of lack of written description.

Claim1 – 3 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the **enablement requirement**. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to **make and/or use** the invention.

In making a determination as to whether an application has met the requirements for enablement under 35 U.S.C. 112 ¶ 1, the courts have put forth a series of factors. See, In re Wands, 8 USPQ2d 1400, at 1404 (CAFC 1988); and Ex Parte Forman, 230 U.S.P.Q. 546 (BPAI 1986). The factors that may be considered include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. Id. While it is not essential that every factor be examined in detail, those factors deemed most relevant should be considered.

The requirement for enablement has not been met in this case for several reasons. There is very little guidance provided in the specification that would allow someone skilled in the art to be able to make and use the invention. The specification does not give any working examples of the inventions that would provide specific amounts of each ingredient or the steps involved in making the composition. There is no scientific information given regarding these ingredients, nor are any references cited. The intended use of composition stated in the claims for weight loss and "lowering the acidity of the body" is not supported by any evidence of these effects in the specification. It is merely asserted by the Applicant. No proposed mechanism by which this

invention would accomplish these results is discussed. Furthermore, there is no description of any testing of the composition to demonstrate that it produces the intended results.

Therefore it would require undue experimentation without a reasonable expectation of success for one of skill in the art to make and/or use the invention as claimed.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1 – 3 are rejected as failing to define the invention in the manner required by 35 U.S.C. 112, second paragraph due to being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The claim(s) are narrative in form and replete with indefinite and functional or operational language. The structure which goes to make up the device must be clearly and positively specified. The structure must be organized and correlated in such a manner as to present a complete operative device. The claim(s) must be in one sentence form only.

Please not that the term "claim" in relation to patent practice is not an assertion of the possible merits of an invention. Claims are intended to specifically describe the invention as a whole. Dependent claims are used to further define various aspects of the invention. The claims are how the Applicant defines the area of intellectual property for which they are seeking coverage.

Note the format of the claims in Fox et al, a patent that has been cited as art below

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1 – 3 are rejected under 35 U.S.C. 103(a) as being unpatentable over Winemiller (Honey Mustard dressing, available on the internet as of November 2001) in view of Fox et al. (US 5,215,769).

Although the claims are exceedingly unclear for reasons given in the above 35 U.S.C. 112, second paragraph rejection, it would appear that the invention is directed towards a composition consisting of garlic powder, apple cider vinegar containing calcium carbonate or other neutralizing buffer, honey and olive oil with an intended use of weight loss and lowering the acidity of the body. Applicant should note that since the invention is a composition, the intended uses are not limiting in regards to applying prior art, i.e., the prior art does not have to have those uses.

Winemiller describes a recipe for honey mustard salad dressing that comprises, among other things, olive oil, cider vinegar (which is known in the art to be synonymous with apple cider vinegar), honey and garlic salt, which is known by one skilled in the art to contain salt and garlic powder. Winemiller does not expressly teach adding calcium carbonate or other neutralizing buffer to the cider vinegar.

Fox et al. teach adding one of various calcium sources such as calcium carbonate to vinegar used within a salad dressing so as to beneficially provide calcium fortification to the salad dressing (see entire document including col 3, lines 7-14).

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to add a calcium source (such as calcium carbonate) to the cider vinegar used within the salad dressing taught by Winemiller based upon the beneficial teachings of Fox et al. with respect to providing calcium fortification to such a salad dressing, as discussed above. The adjustment of particular conventional working conditions (e.g., adding particular amounts of such ingredients to a salad dressing) is deemed merely a matter of judicious selection and routine optimization which is well within the purview of the skilled artisan.

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Conclusion

No claims are allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Heather Anderson whose telephone number is (571) 270-3051. The examiner can normally be reached on Monday-Thursday, 7:30 AM-5:00 PM, ALT. Friday, EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mary Mosher can be reached on (571) 272-0906. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

CHRISTOPHER R. TATE
PRIMARY EXAMINER

Notice of References Cited	Application/Control No. 10/620,915	Applicant(s)/Patent Under Reexamination PETICCA, ANDREA LYNN	
	Examiner Heather Anderson	Art Unit 1609	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,215,769	06-1993	Fox et al.	426/74
*	B	US-7,037,519	05-2006	Humphrey, Donna	424/450
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Winemiller, Honey Mustard, Recipesource.com [online], posted November 23, 2001 [retrieved on 2007-06-06]. Retrieved from the Internet: <URL: http://.recipesource.com/side-dishes/dressings/02/rec0290.html>.
	V	Internet Archive search result showing oldest posting date of Recipesource.com webpage, Internet Archive Wayback Machine [online], [retrieved on 2007-06-06]. Retrieved from the Internet: <URL: http://web.archive.org/web/*/http://www.recipesource.com/side-dishes/dressings/02/rec/0290.html>
	W	Brighenti et al., "Effects of netralized and native vingar on blood glucose and acetate responses to a mixed meal in healthy subjects," European Journal of Clinical Nutrition, April 1995, Volume 49(4), pages 242-247.
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

RecipeSource : Side Dishes : Salad Dressing Recipes : "Houston's" Honey Mustard Dressing

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---------- Recipe via Meal-Master (tm) v8.02

      Title: "HOUSTON'S" HONEY MUSTARD DRESSING
 Categories: Salads, Dressings
      Yield: 1 servings

     1 c  Olive oil
   1/4 c  Cider vinegar
 1 1/8 c  Honey
   1/2    Jar Dijon mustard
     1 ds Garlic salt (generous)
   3/4 c  Mayonnaise

  Mix all ingredients together with wire whisk until
  mayo can't be seen. Refrigerate several hours.

  Great also as a dip with chicken fingers and as a
  spread on ham or turkey club sandwiches.

  FROM:    BARBARA WINEMILLER   (HDJF80A)

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INTERNET ARCHIVE
WaybackMachine

Enter Web Address: http:// All Take Me Back Adv. Search Compare Archive Pages

Searched for http://www.recipesource.com/side-dishes/dressings/02/rec0290.html

40 Results

Note some duplicates are not shown. See all.
* denotes when site was updated.

Search Results for Jan 01, 1996 - Jun 06, 2007

1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
0 pages	0 pages	0 pages	0 pages	0 pages	1 pages	2 pages	3 pages	8 pages	18 pages	6 pages	0 pages
					Nov 23, 2001 *	Oct 15, 2002	Aug 02, 2003 *	Apr 06, 2004	Jan 13, 2005 *	Jan 01, 2006	
						Dec 26, 2002 *	Oct 15, 2003	Jun 20, 2004	Mar 01, 2005 *	Jan 04, 2006	
							Dec 04, 2003 *	Jul 22, 2004 *	Mar 03, 2005	Feb 02, 2006 *	
								Aug 12, 2004	Mar 06, 2005	Feb 03, 2006	
								Aug 13, 2004	Apr 25, 2005	Feb 05, 2006	
								Oct 20, 2004	Jun 04, 2005 *	Feb 21, 2006	
								Nov 18, 2004	Jun 20, 2005		
								Dec 04, 2004 *	Jul 05, 2005 *		
									Aug 05, 2005		
									Aug 26, 2005		
									Aug 30, 2005		
									Nov 04, 2005 *		
									Nov 07, 2005		
									Nov 07, 2005 *		
									Nov 26, 2005		
									Dec 05, 2005 *		
									Dec 11, 2005		
									Dec 23, 2005		

European Journal of Clinical Nutrition (1995) 49, 242–247

Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects*

F Brighenti[1], G Castellani[2], L Benini[2], MC Casiraghi[1], E Leopardi[1], R Crovetti[1] and G Testolin[1]

[1]*DiSTAM (Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche), Sezione Nutrizione, University of Milan, Via Celoria 2, 20133 Milan; and* [2]*Divisione Gastroenterologia, Centro Ospedaliero Clinicizzato Valeggio S/M, University of Verona, Italy*

Objective: To investigate the influence of sodium acetate and acetic acid from vinegar on blood glucose and acetate response to a mixed meal in healthy subjects.

Design: Five healthy subjects consumed in random order six test meals consisting of 100 g of sliced lettuce dressed with olive oil (Blank), olive oil plus 1 g acetic acid in the form of vinegar (AcOH), or olive oil plus sodium acetate in the form of vinegar neutralized to pH 6.0 with sodium bicarbonate (AcNa). On three occasions test meals were followed by a challenge consisting of 50 g carbohydrate portions of white bread (Bread). Glucose and acetate concentrations were measured in arterialized capillary blood before and until 95 min after the meals. Ultrasonography was performed in four other subjects to measure gastric emptying times after AcOH + Bread and AcNa + Bread.

Results: Blood acetate response over 95 min was markedly reduced after AcOH and AcOH+Bread meals compared to AcNa and AcNa + Bread. Similarly, the glucose response was depressed by 31.4% ($P = 0.0228$) after AcOH+Bread with respect to AcNa + Bread and Blank + Bread. No difference was observed between gastric emptying times after AcOH + Bread and AcNa + Bread.

Conclusions: The results suggest that oral acetic acid and acetate might have a different effect on acetataemia and that a limited dose of vinegar, in the form of salad dressing, is sufficient to influence significantly the glycaemic response to a mixed meal in normal subjects by a mechanism related to acidity but not to gastric emptying.

Sponsorship: National Research Council of Italy (CNR) targeted Project 'Prevention and Control of Disease Factors', Subproject 'Nutrition'; Contract no. 9200226.PF11.

Descriptors: acetic acid, blood acetate, blood glucose, carbohydrates, sodium acetate, vinegar

Introduction

Various sources contribute to human plasma acetate levels: endogenous metabolism of glucose and fatty acids (Knowles *et al.*, 1974), oxidation of dietary alcohol (Yki-Jarvinen, Koivisto & Ylikahri, 1988), colonic absorption of acetate derived from bacterial degradation of undigested carbohydrates (Cummings, 1983; Akanji *et al.*, 1989*a*) and dietary acetic acid and its salts. Among the many postul increased levels of plasma acetat lipolysis with a consequent fall free fatty acids (FFA) is suppo mental evidence (Crouse *et al.*, *al.*, 1989*b*). It has been suggeste an action on the glucose/fa (Randle, Garland & Hales, 196 sion of FFA by acetate cou positive effect on glucose me mined by diets rich in undig drates, whose breakdown pro rise in acetataemia (Wolever *e*

In effect, it has been est fermentable load made availa daily in normal individual 300–800 mmol of volatile fat the molar ratio 60:20:20 for nate, and butyrate, respec Wolever & Jeejebhoy, 1990 acids are well absorbed by the case of acetate, only partiall liver during the first pass, t peripheral blood. However, tion is a relatively slow pro delayed excursion in the acetate even if the amounts o are relatively important.

Other dietary manoeuvre ingestion of acetic acid or s raise plasma acetate levels potentially affect glucose concomitantly to a carbohyd data in this regard are con and colleagues (1988*a*,*b*) acetate given by mouth had tolerance and turnover in Ebihara & Nakajima's stu response was flattened i response was flattened in h a carbohydrate load contain form of vinegar.

As vinegar dressing is dietary habit, we studied realistic amount of vineg dietary carbohydrate coing mixed meal, on blood gluc in healthy volunteers. In istered vinegar in both na forms to evaluate the between the effect of o sodium acetate on plasma levels.

Correspondence to: Dr Furio Brighenti.
*Results presented in part at the Ninth International Symposium on Diabetes and Nutrition, 13–15 June 1991, Uppsala, Sweden.
Received 24 February 1994; revised 3 November 1994; accepted 10 November 1994

its salts. Among the many postulated effects of increased levels of plasma acetate, inhibition of lipolysis with a consequent fall in circulating free fatty acids (FFA) is supported by experimental evidence (Crouse *et al.*, 1968; Akanji *et al.*, 1989*b*). It has been suggested that, through an action on the glucose/fatty acid cycle (Randle, Garland & Hales, 1963), the suppression of FFA by acetate could explain the positive effect on glucose metabolism determined by diets rich in undigested carbohydrates, whose breakdown produces a chronic rise in acetataemia (Wolever *et al.*, 1989).

In effect, it has been estimated that the fermentable load made available to the colon daily in normal individuals would yield 300–800 mmol of volatile fatty acids daily in the molar ratio 60:20:20 for acetate, propionate, and butyrate, respectively (Royall, Wolever & Jeejebhoy, 1990). Volatile fatty acids are well absorbed by the colon and, in the case of acetate, only partially cleared by the liver during the first pass, thus reaching the peripheral blood. However, colonic fermentation is a relatively slow process, resulting in delayed excursion in the levels of plasma acetate even if the amounts of acetate absorbed are relatively important.

Other dietary manoeuvres such as direct ingestion of acetic acid or sodium acetate can raise plasma acetate levels acutely and could potentially affect glucose tolerance if given concomitantly to a carbohydrate load, although data in this regard are contrasting. Sheppach and colleagues (1988*a*,*b*) found that sodium acetate given by mouth had no effect on glucose tolerance and turnover in man, whereas in Ebihara & Nakajima's study (1988) glucose response was flattened in rats and insulin response was flattened in healthy humans after a carbohydrate load containing acetic acid in the form of vinegar.

As vinegar dressing is a common Italian dietary habit, we studied the influence of a realistic amount of vinegar, with or without dietary carbohydrate coingested as a part of a mixed meal, on blood glucose and acetate levels in healthy volunteers. In addition, we administered vinegar in both native and neutralized forms to evaluate the possible difference between the effect of oral acetic acid and sodium acetate on plasma glucose and acetate levels.

Methods

Subjects and meals

Five healthy volunteers, four male and one female, 37 ± 3 years of age (mean ± s.e.m.), 98 ± 4% ideal body weight, were studied on a total of six separate occasions in the morning after an overnight fast. On three occasions the subjects were given a meal consisting of iceberg lettuce, purchased the same morning at the local market and sliced in strips of about 2 × 0.5 cm, and dressed with 10 g of refined olive oil and 1.5 g NaCl (Blank), or olive oil plus either acetic acid in the form of white vinegar (AcOH), or sodium acetate in the form of neutralized vinegar (AcNa). For AcOH meals, 20 ml vinegar (5% acetic acid, Allen's Pure White Vinegar, Toronto, Ontario) was given as a vinaigrette sauce made with 10 g olive oil and 1.5 g NaCl. For AcNa meals, 20 ml vinegar was neutralized (final pH about 6.0) with a stoichiometric equivalent (1.5 g) of food-grade sodium bicarbonate, and the vinaigrette sauce was prepared without salt. On three occasions meals were followed by a challenge consisting of a 50 g carbohydrate portion of white bread (Bread), consumed immediately after the salad. The time allowed for meal consumption was 5 min (salad alone) or 15 min (5 for salad plus 10 for bread); any vinaigrette sauce left in the bowl was drunk before starting to eat the bread. Meals were given in random order at intervals of at least 4 days (mean 7 days).

Arterialized capillary blood samples were obtained by pricking warmed fingers before (time −5 min) and at the end (time 0 min) of the salad meal, and thereafter every 15 min for 1½ h. Four drops of blood were placed into fluocitrate tubes for glucose analysis with an automatic analyser (model 27AP, Yellow Springs Instruments, Yellow Springs, OH). One hundred µl of plasma were analysed for acetate by high-performance liquid chromatography (HPLC) (Wolever, Brighenti & Jenkins, 1988) after perchloric deproteinization and vacuum distillation (Tollinger, Vreman & Weiner, 1979). Incremental values were calculated individually by subtracting the fasting value (time −5 min) for acetate and the post-salad (time 0 min) values for glucose at each time point. Incremental areas under the curve (IAUC) were calculated according to Wolever & Jenkins (1986).

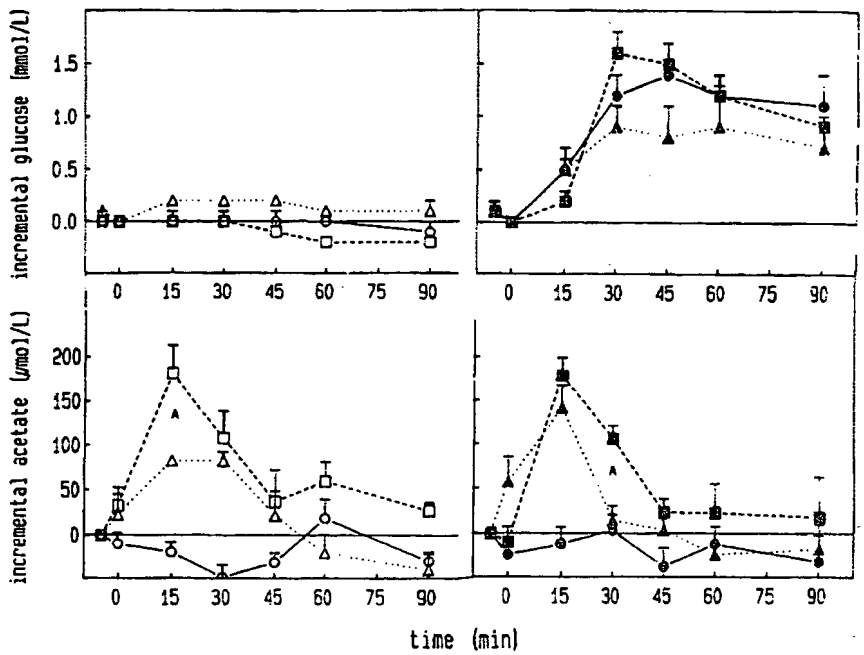

Figure 1 Changes in plasma glucose (top) and plasma acetate (bottom) following ○, Blank, △, AcOH, and □, AcNa meal consumed with (closed symbols) and without (open symbols) 50 g carbohydrates from bread. Values expressed as mean +s.e.m. A = significant ($P < 0.05$) difference between AcOH and AcNa.

To exclude the possibility that differences in acidity could influence gastric emptying of carbohydrates, four subjects, three male and one female matched for age and body weight to four of the volunteers, were given ACOH+Bread and AcNa + Bread meals in random order on two different days, and their gastric antrum diameters were measured by real time ultrasonography before the meal, immediately after it and every 15 min until the premeal values were observed again.

All measurements were performed by the same operator (G.C.). The mean of three readings was calculated during interperistaltic relaxation at each time point. The antral cross-sectional area was calculated assuming an elliptical shape. According to Bolondi *et al.* (1985), antral cross-sectional area closely reflects the amount of food remaining in the stomach. The total emptying time was defined as the time required for the return of postprandial cross-sectional area to the fasting values. It was calculated by the regression equation of antral area with time, considering only the time interval from the maximal value to the return to baseline.

Statistical analysis

Differences among treatments were assessed by analysis of variance (ANOVA), blocked by subjects, followed by the Newmann–Keuls range test for multiple comparisons. Differences between gastric emptying values were evaluated by paired Student's *t*-test (two-tailed). Subjects were fully informed of the study protocol, which was in accord with the ethical standards of the University of Milan Ethics Committee.

Results

Meals were considered palatable and were consumed completely within the scheduled time. Fasting glucose and acetate averaged 4.41 ± 0.05 mmol/l and 157.9 ± 12.0 μmol/l, with no significant differences among treatments. Plasma acetate remained around the baseline values after the two meals without vinegar

Table 1 Individual and mean (±s.e
acetate incremental areas under the
(μmol.min/l) following AcOH and
without bread challenge

	–Bread	
	AcOH	*AcNa*
1	4889	8800
2	2844	4421
3	2625	10610
4	2821	7582
5	3595	4022
Mean	3355	7084
s.e.m.	418	1267

ANOVA:
effect of treatment: $F = 5.598$;
effect of neutralization: $F = 18.$
effect of bread: $F = 0.157$; $P =$

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vinegar (AcOH and AcC
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15 min and then returned t
between 30 and 60 min
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significant ($P < 0.05$) decre
30 min compared to all oth
AcNa produced both a h
ataemia and a slower ret
value compared to AcOH.

Table 2 Individual and mean (±s
glucose incremental areas under t
(mmol.min/l) following Blank, Ac
with bread challenge

	Blank
1	84.0
2	121.5
3	52.5
4	91.5
5	96.8
Mean	89.3
s.e.m.	11.1

ANOVA: effect of treatment $F =$
Range test: AcOH was different
< 0.05).

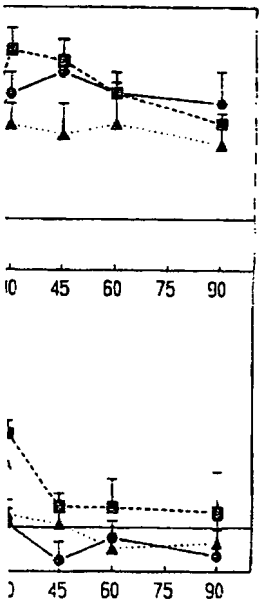

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Table 1 Individual and mean (±s.e.m.) values of plasma acetate incremental areas under the −5:90 min curve (μmol.min/l) following AcOH and AcNa meals with and without bread challenge

	−Bread		+Bread	
	AcOH	*AcNa*	*AcOH*	*AcNa*
1	4889	8800	2326	5994
2	2844	4421	4398	4949
3	2625	10610	5662	11116
4	2821	7582	2110	3622
5	3595	4022	2073	5322
Mean	3355	7084	3314	6200
s.e.m.	418	1267	730	1288

ANOVA:
effect of treatment: $F = 5.598$; $P = 0.0123$;
effect of neutralization: $F = 18.04$; $P = 0.0008$;
effect of bread: $F = 0.157$; $P = 0.7024$.

(Blank and Blank + Bread), whereas with vinegar (AcOH and AcOH + Bread) and neutralized vinegar (AcNa and AcNa + Bread) plasma acetate rose about twofold within 15 min and then returned to the baseline value between 30 and 60 min (Figure 1). Without bread AcNa induced a significant ($P < 0.05$) increase in acetataemia at 15 min compared to AcOH, whereas with bread AcOH induced a significant ($P < 0.05$) decrease in acetataemia at 30 min compared to all other meals. Moreover, AcNa produced both a higher rise in acetataemia and a slower return to the baseline value compared to AcOH, whether bread was

Table 2 Individual and mean (±s.e.m.) values of plasma glucose incremental areas under the 0:90 min curve (mmol.min/l) following Blank, AcOH, and AcNa meals with bread challenge

	Blank	*AcOH*	*AcNa*
1	84.0	70.5	96.0
2	121.5	97.5	89.3
3	52.5	17.1	81.0
4	91.5	60.8	88.6
5	96.8	60.7	92.3
Mean	89.3	61.3	89.4
s.e.m.	11.1	12.9	2.5

ANOVA: effect of treatment $F = 6.293$; $P = 0.0228$.
Range test: AcOH was different from Blank and AcNa ($P < 0.05$).

Table 3 Individual and mean (±s.e.m.) gastric emptying times (min) following AcOH and AcNa meals with bread challenge

	AcOH	*AcNa*
1	55.0	69.9
2	55.8	60.6
3	73.5	74.8
4	113.0	99.0
Mean	74.3	76.1
s.e.m.	13.6	8.2

P = n.s.

eaten or not. Consequently, plasma acetate IAUC were significantly higher after acetate than after acetic acid meals (Table 1). On the contrary, statistical analysis showed that the presence of bread had no effect on incremental acetataemia. The AcOH + Bread meal elicited a flatter glucose response (Figure 1), resulting in a reduction of about 30% in the mean glucose IAUC compared to the Blank + Bread meal (Table 2). However, when vinegar was neutralized, the glucose response was similar to that observed after the Blank meal. The differences were confirmed by ANOVA of glucose IAUC values of the three meals that included the bread challenge ($F = 6.293$, $P = 0.0228$, Newmann–Keuls test $P < 0.05$ for AcOH + Bread compared to AcNa + Bread and Blank + Bread). Finally, no differences were observed in gastric emptying times after the two meals containing vinegar or neutralized vinegar followed by the bread challenge (74.3 ± 13.6 min for AcOH + Bread *vs* 76.1 ± 8.2 min for AcNa + Bread; $t = 0.29$, $P = 0.789$) (Table 3).

Discussion

Acetate derived from colonic fermentation has been suggested as a possible mechanism by which fermentable dietary fibre can reduce postprandial glycaemia. In this study, ingestion of 16 mmol of acetic acid from vinegar with a carbohydrate-rich food (bread) flattened postprandial glycaemia in healthy subjects. This effect disappeared when the pH was corrected to 6.0 with sodium bicarbonate. Moreover, blood acetate IAUC were markedly reduced after acetic acid compared to acetate, whether bread was added or not. Similarly, Sheppach *et*

al. (1988*a*) found that acetate (15 mmol every 15 min) given by mouth had no effect on glucose tolerance after a drink of 50 g glucose, whereas Ebihara & Nakajima (1988) observed a 20% decrease of the area under the plasma insulin curve in healthy volunteers after a drink of 53 g sucrose plus 60 ml of strawberry vinegar, and a significant reduction in plasma glucose in rats given 10% corn starch solution with 2% acetic acid intragastrically compared with control meals.

These findings are puzzling, as they seem to indicate that sodium acetate and acetic acid may behave differently in influencing both glucose and acetate absorption and disposal. In our study, differences in glucose IAUCs were due not only to lower peak values but to an overall reduction in glycaemia after acetic acid. However, after 90 min from the beginning of the bread challenge, the values were still well above the fasting values for all the meals. It is thus possible that in the missing part of the curve the differences in IAUCs could have been compensated by a more rapid fall in the glycaemia after the meals containing no vinegar and neutralized vinegar, as normally seen in high glycaemic index compared to low glycaemic index foods (Wolever, 1990). However, this does not negate the potentially positive effect of native vinegar in controlling glucose response.

A possible explanation for the observed differences in apparent glucose tolerance could be a change in gastric emptying pattern of the meals due to either differences in pH or to the presence of acetic acid, as acidity and fatty acids are known to affect stomach motility through duodenal receptor-mediated mechanisms (Hunt, 1963; Hunt & Knox, 1972). In rats, when vinegar and ethanol were given simultaneously by mouth, vinegar delayed the disappearance of ethanol from the stomach (Mochizuki *et al.*, 1987). However, comparing the effect on gastric emptying of fatty acids of different chain length, Hunt & Knox (1968) demonstrated clearly that little to no slowing is brought about by fatty acids with a chain length of 2 to 10 carbon atoms. Moreover, the similarity in gastric emptying times of AcOH + Bread and AcNa + Bread meals as assessed by ultrasound measurement of antral areas seems to rule out the possibility that acidity or the presence of sodium acetate could have been responsible for a major difference between the two meals in the rate of delivery of carbohydrates to the duodenum.

Another possible explanation could be due to differences in the cephalic phase response to the meals, and specifically to that containing native vinegar since AcNa and Blank meals gave virtually identical responses. Although none of the volunteers complained about the palatability of the AcOH meal, it is possible that the strong acid feeling of native vinegar could have determined differences in the cephalic phase response, including gastric acid secretion, and perhaps insulin secretion or gastrointestinal hormones which potentiate insulin secretion.

Regarding acetataemia, the early rise and early fall in blood acetate observed by us after vinegar compared to neutralized vinegar, suggests, if anything, a faster absorption of acetic acid with respect to acetate. Acetic acid is easily absorbed from the gastric mucosa at low pH, that is in protonated form; this could partly explain the anticipated absorption observed in the AcOH meal, considering that the presence of sodium acetate could have buffered gastric acidity to some extent. However, it does not explain the lower area under the acetate curve after the meals containing acetic acid compared to those containing sodium acetate, unless there were a rise in plasma acetate from 25–50 μmol/l up to values of 450–500 μmol/l and a subsequent fall to 80–150 μmol/l between the two blood samplings at 0 and 15 min. After acetic acid, there was a further apparent improvement in acetate tolerance, suggested by an early fall in blood acetate at 30 min, when glucose was available simultaneously in the form of bread. However, identical acetate disposal rates were observed after acetate with and without bread. In ruminants, glucose has a permissive role in acetate utilization (Jarret & Filsell, 1961), whereas in normal and diabetic humans impaired acetate tolerance has been noted with glucose and acetate infused i.v. (Akanji & Hockaday, 1990). It is thus possible that the route (intestinal *vs* i.v.) and the form (acetic acid *vs* acetate) of acetate administration could play a role in determining the reciprocal metabolic effects of glucose and acetate, even though the mechanism by which this effect is carried out remains obscure.

In conclusion, this study has shown that: (i) sodium acetate appears later in peripheral blood and induces higher acetataemia than acetic acid; (ii) in normals, blood glucose r
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(ii) in normals, blood glucose response after a carbohydrate challenge is markedly reduced by a small amount of acetic acid but unaffected by sodium acetate given orally; (iii) in normals, blood acetate responses after acetic acid are slightly influenced by coingestion of carbohydrates; (iv) traditional eating habits, such as the use of vinegar in preservation of vegetables and in salad and potato dressings, could have a potential beneficial effect in reducing the overall glycaemic response to a meal containing carbohydrates and fat.

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